AMENDMENTS TO THE CLAIMS

This listing of claims will replace all prior versions, and listings, of claims in the application:

Listing of the Claims

What is claimed:

1. (Currently Amended) A method for multiplex primer-based amplification of a target sequence from a plurality of agents, said target sequence being different for each agent, ~~wherein~~ said method comprising ~~comprises carrying out said amplification in a reaction mixture comprising at least a first and a second pair of target enrichment primers and at least a first pair of target amplification primers, said target amplification primers comprising a FSP and a RSP, said method comprising~~:

   a. carrying out a first amplification reaction for each target sequence to be amplified using

      i) as a template, a nucleic acid from each of said plurality of agents ~~at least one agent~~, said nucleic acid containing said ~~at least one~~ target sequence ~~from said agent~~;

      ii) ~~said~~ a first pair of target enrichment primers hybridizing to said nucleic acid and bracketing said ~~at least one~~ target sequence;

      iii) ~~said~~ a second pair of target enrichment primers hybridizing to said nucleic acid and bracketing said ~~at least one~~ target sequence, said second pair of target enrichment primers being located proximate to said ~~at least one~~ target sequence and one of ~~each of~~ the second pair of target enrichment primers comprising at its 5' end a ~~super primer~~ binding tag corresponding to the sequence of one of a pair of ~~the~~ target amplification primers and the other of the second pair of target enrichment primers comprising at its 5' end a binding tag corresponding to the sequence of the other of said pair of target amplification primers ; and

      iv) amplification reagents and conditions for said first amplification reaction such that the first amplification reaction generates a plurality first amplification products, wherein at least a portion of the first amplification products contain said target sequence and at least one complement of the ~~super primer~~ binding tag for one of said target enrichment primers ~~tags~~ thereby forming at least one ~~super primer~~ binding site for at least one of said target enrichment primers; and

   b. carrying out a second amplification reaction for each target sequence to be amplified using

i) as a template, said portion of the first amplification products containing said at least one ~~super primer~~ binding site for at least one of said target enrichment primers;

ii) said first pair of target amplification primers binding to their corresponding ~~super primer~~ binding sites on said portion of first amplification products; and

iii) amplification reagents and conditions for said second amplification reaction such that the second amplification reaction generates a plurality second amplification products containing the ~~at least one~~ target sequence.

2. The method of claim 1 where said first pair of target enrichment primers comprises a R_o and a F_o primer, said second pair of target enrichment primers comprises a F_i and a R_i primer and said first pair of target amplification primers comprises a FSP and a RSP.

3. The method of claim 2 where said super primer binding tag on F_i is identical to the sequence of the FSP such that the FSP binds the complement of the super primer binding tag on said F_i primer and the super primer binding tag on R_i is identical to the sequence of the RSP such that the RSP binds the complement of the super primer binding tag on said R_i primer.

4. The method of claim 1 where the length of each of the first pair of target enrichment primers is selected from the group consisting of: 10-40 nucleotides, 10-30 nucleotides and 10-20 nucleotides.

5. The method of claim 1 where the length of each of the second pair of target enrichment primers is selected from the group consisting of: 10-40 nucleotides, 10-30 nucleotides and 10-20 nucleotides.

6. The method of claim 1 where the length of each of the first pair of target enrichment primers is 10-20 nucleotides and the length of each of the second pair of target enrichment primers is 30 to 40 nucleotides.

7. The method of claim 1 where the length of each of the first pair of target amplification primers is 10-20 nucleotides and the length of each of the second pair of target enrichment primers is 30 to 40 nucleotides.

8. The method of claim 1 where the target enrichment primers are present at a low concentration and the target amplification primers are present at a high concentration.

9. The method of claim 8 where said low concentration is a concentration of 0.002 μM to 0.2 μM and said high concentration is a concentration of 0.2 μM to 1.0 μM.

10. The method of claim 1 where the target enrichment primers are present at a concentration that is not sufficient for exponential amplification of the target sequence and the target

amplification primers are present at a concentration that is sufficient for exponential amplification of the target sequence.

11. The method of claim 1 where each of the target enrichment primers is used at the same concentration.
12. The method of claim 1 where at least one of the target enrichment primers is used at a higher concentration than the other target enrichment primers.
13. The method of claim 1 where each of the target amplification primers is used at the same concentration.
14. The method of claim 1 where at least one of the target amplification primers is used at a higher concentration than the other target amplification primer.
15. The method of claim 14 where said target amplification primer at said higher concentration comprises a means for detection.
16. The method of claim 1 where the conditions for said first amplification reaction comprise at least two complete cycles of a target enrichment process and the conditions for said second amplification process comprise at least two complete cycles of a target amplification process.
17. The method of claim 16 where the target enrichment process comprises the following conditions for amplification: 0.5 to 1 minute at 92-94^{0}C, 1-2.5 minutes at 50-55^{0}C and 0.5 to 1 minute at 70-72^{0}C and the target amplification process comprises the following conditions for amplification: 15 to 30 seconds at 94^{0}C, 15 to 30 seconds at 50-55^{0}C and 15 to 30 second at 72^{0}C.
18. The method of claim 1 where the conditions for said first amplification reaction comprise at least two complete cycles of a target enrichment process and at least two complete cycles of a selective amplification process and the conditions for said second amplification process comprise at least two complete cycles of a target amplification process.
19. The method of claim 18 where the target enrichment process comprises the following conditions for amplification: 0.5 to 1 minute at 92-94^{0}C, 1-2.5 minutes at 50-55^{0}C and 0.5 to 1 minute at 70-72^{0}C, the selective amplification process comprises the following conditions for amplification: 15 to 30 seconds at 92-94^{0}C, 1 to 2 minutes at 70-72^{0}C and the target amplification process comprises the following conditions for amplification: 15 to 30 seconds at 94^{0}C, 15 to 30 seconds at 50-55^{0}C and 15 to 30 second at 72^{0}C.

20. (Currently Amended) The method of claim 19 where the selective amplification is biased toward the production of first amplification products containing the ~~super primer~~ binding site for said target enrichment primers.
21. The method of claim 19 where the length of each of the first pair of target enrichment primers is 10-20 nucleotides and the length of each of the second pair of target enrichment primers is 30 to 40 nucleotides.
22. The method of claim 1 further comprising three or more pairs of target enhancement primers.
23. (Currently Amended) The method of claim 1 further comprising two or more pairs of target amplification primers.
24. (Currently Amended) The method of claim 1 [[24]] where said agent is selected from the group consisting of: a virus and a bacteria.
25. (Currently Amended) The method of claim 24 where said ~~agent is a~~ virus is selected from the group consisting of: adenovirus, influenza A, influenza B, parainfluenza type 1, parainfluenza type 3, and respiratory syncytial virus, SARS, and enterovirus, including, coxsackie virus A, coxsackie virus B, rhinovirus, and echovirus.
26. (Currently Amended) The method of claim 24 where said ~~agent is a~~ bacteria is selected from the group consisting of" *Mycoplasma* species and *Chlamydia* species.
27. (Currently Amended) The method of claim 24 where said agent is selected by the appropriate design of the first and second pair of target enrichment primers.
28. The method of claim 1 where at least one of said forward or reverse super primers further comprises a means for detection.
29. (Currently Amended) The method of claim 28 where said means for detection is selected from the group consisting of: a chemical element, an enzymatic element, a fluorescent element, or a radiolabel element.
30. The method of claim 1 further comprising detecting said target sequence.
31. The method of claim 30 where the detection method is a direct detection method.
32. (Currently Amended) The method of claim 30 where said detection method comprises:
    a. providing ~~at least one set of~~ a detection oligonucleotide for each target sequence to be detected, each detection oligonucleotide ~~set of detection oligonucleotide having a nucleotide sequence capable of binding a specific target sequence in said amplification products and~~ comprising a first means for signal generation;

b. contacting and incubating said detection oligonucleotide ~~oligonucleotides~~ with said second amplification products;

c. stimulating said first means for signal generation to produce a first signal; and

d. detecting said first signal.

33. (Currently Amended) The method of claim 32 where said first signal is unique for each target sequence to be detected ~~said agent~~ and said first signal is used to identify said agent.

34. The method of claim 32 where said means for first signal generation is a fluorescent label, a chemical label, an enzymatic label, or a radiolabel.

35. The method of claim 32 where said means for first signal generation is a fluorescent microsphere.

36. The method of claim 30 where said method is an indirect detection method.

37. (Currently Amended) A method for multiplex primer-based amplification of a target sequence from a plurality of agents, said target sequence being different for each agent, ~~of at least one target sequence, wherein~~ said method ~~comprises carrying out said amplification in a reaction mixture comprising at least a first pair of target enrichment primers and at least a first pair of target amplification primers, said target amplification primers comprising a FSP and a RSP, said method~~ comprising:

a. carrying out a first amplification reaction for each target sequence to be amplified using

i) as a template, a nucleic acid from each of said plurality of agents ~~at least one agent~~, said nucleic acid containing said ~~at least one~~ target sequence ~~from said agent~~;;

ii) ~~said~~ a first pair of target enrichment primers hybridizing to said nucleic acid and bracketing said target sequence, each of the first pair of target enrichment primers comprising at its 5' end a ~~super primer~~ binding tag corresponding to the sequence of one of a pair of ~~the~~ target amplification primers and the other of the second pair of target enrichment primers comprising at its 5' end a binding tag corresponding to the sequence of the other of said pair of target amplification primers; and

iii) amplification reagents and conditions for said first amplification reaction such that the first amplification reaction generates a plurality first amplification products, wherein at least a portion of the first amplification products contain said target sequence and at least one complement of the ~~super primer~~ binding tag for one of said target enrichment primers ~~tags~~ thereby forming at least one ~~super primer~~ binding site for at least one of said target enrichment primers; and

b. carrying out a second amplification reaction for each target sequence to be amplified using

i) as a template, said portion of the first amplification products containing said at least one ~~super primer~~ binding site for at least one of said target enrichment primers;

ii) said first pair of target amplification primers binding to their corresponding ~~super primer~~ binding sites on said portion of first amplification products; and

iii) amplification reagents and conditions for said second amplification reaction such that the second amplification reaction generates a plurality second amplification products containing the target sequence.

38-68 (Cancelled)

69. (Currently Amended) A method of diagnosing the presence of a disease agent in a subject, said method comprising:

a. providing a sample from said subject in need of said diagnosis, said sample suspected of containing said disease agent;

b. isolating a nucleic acid from said sample, said nucleic acid containing a target sequence from said disease agent;

c. subjecting said nucleic acid to the primer-based amplification method of claim ~~any of claims~~ 1 or 37;

d. detecting said target sequence from said disease agent.

70-78 (Cancelled)

79. (Currently Amended) A method for differentially diagnosing the presence of a disease agent and a secondary disease agent in a subject, said method comprising:

a. providing a sample from said subject in need of said diagnosis, said sample suspected of containing said disease agent or said secondary disease agent;

b. isolating a nucleic acid from said sample, said nucleic acid containing a target sequence from said disease agent or secondary disease agent or both;

c. subjecting said nucleic acid to the primer-based amplification method claim ~~of any of claims~~ 1 or 37 [[38]];

d. detecting said target sequence from said disease agent or secondary disease agent or both.

80-88 (Cancelled)